EFFECT OF VARIOUS IRRIGANTS ON WETTABILITY AND PUSH-OUT BOND STRENGTH OF AN EPOXY RESIN-BASED ROOT CANAL SEALER – AN IN VITRO STUDY

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CERTIFICATE

This is to certify that this dissertation titled **"Effect of various irrigants on wettability and push-out bond strength of an epoxy resin-based root canal sealer -an in vitro study"** is a bonafide record of work done by **Dr.KEERTHANA C** under my guidance and to my satisfaction during her postgraduate study period, 2014 – 2017. This dissertation is submitted to **THE TAMILNADU Dr. M.G.R. MEDICAL UNIVERSITY**, in partial fulfillment for the award of the degree of Master of Dental Surgery in Conservative Dentistry and Endodontics, Branch IV. It has not been submitted (partially or fully) for the award of any other degree or diploma.

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ABSTRACT

AIM OF THE STUDY:

The aim of this in vitro study was to evaluate the effect of various final irrigation regimen on the wettability and push out bond strength of an epoxy resin root canal sealer AH plus.

METHODOLOGY:

To assess wettability, 20 premolars were cross-sectioned to prepare 40 dentin discs. Dentin discs were then divided randomly into 4 groups (n = 10) depending on the irrigation regimen: Group I: 5 ml of 3% NaOCl; Group II: 5 ml of 3% NaOCl + 5 ml of 17% EDTA; Group III: 5 ml of 3% NaOCl + 5 ml of 10%citric acid; Group IV: 5 ml of 3% NaOCl + 5 ml of Q mix. Irrigation regimens were performed for 1 min. Each specimen was placed inside a Dynamic Contact Angle Analyzer. A controlled-volume droplet of sealer was placed on each specimen and the static contact angle was analyzed.

To assess push out bond strength, eighty extracted premolars with single canal were collected and decoronated at cementoenamel junction (CEJ). Teeth were randomly divided into four groups based on irrigation regimen similar to grouping in wettability as mentioned above. Biomechanical preparation was performed with ProTaper Universal NiTi rotary instrument. Obtuartion was done with AH plus sealer and guttapercha cones by cold lateral condensation. The dislocation resistance was assessed by using push-out bond strength test. The data was statistically analyzed.

RESULTS:

The contact angles of group 4 (3% NaOCl + Q mix) was significantly less compared to group 1(3% NaOCl), group2 (3% NaOCl + 17% EDTA) and group 3(3% NaOCl + 10% Citric acid). The contact angles of group 2 (3% NaOCl + 17% EDTA) and group 3 (3% NaOCl + 10% Citric acid) were significantly larger than group 1 (3% NaOCl). However, there was no significant difference between the contact angles produced by group 2 (3% NaOCl + 17% EDTA) and group 3 (3% NaOCl + 10% Citric acid).

The push out bond strength of group 2 (3% NaOCl + 17% EDTA), group 3 (3% NaOCl + 10% Citric acid) and group 4 (3% NaOCl + Q mix) were significantly higher than that of group 1 (3% NaOCl) . However there was no significant difference between group 2 (3% NaOCl + 17% EDTA), group 3 (3% NaOCl + 10% Citric acid) and group 4 (3% NaOCl + Q mix).

CONCLUSION:

Within the limitations of this in vitro study, QMix enhanced the wettability of AH plus root canal sealer to dentin. Chelating agent enhanced the bond strength of the AH Plus to root dentin. However there was no significant difference in bond strength of AH plus when root canal was irrigated with QMix compared to EDTA and Citric acid.

CONTENTS

Introduction

INTRODUCTION

Adhesion is a clinically favorable property of root canal sealers. Adhesion of an endodontic sealer is defined as its capacity to adhere to the root canal walls and promote the union of gutta-percha cones to each other and to the dentin. [1,2]

Contemporary endodontics has seen an unequalled advance in technology and materials. These advances aim at improving the standard of care in endodontics. Adhesive root filling materials which were introduced to adhere to coronal dentine, could minimize leakage by increasing the seal between the core root filling material and the root canal walls.[3] AH Plus, is an epoxy based root canal sealer. It is characterized by very good mechanical properties, high radiopacity, reduced polymerization shrinkage, low solubility and not the least, a high degree of stability on storage. Studies have shown that AH plus has higher bond strength to root dentine than methacrylate sealers.[4,5]

Interestingly, irrigation protocols seem to influence the adhesion of sealers to root dentine.[6,7] Adhesion of AH Plus to root dentine is associated to covalent bonds between epoxide rings and the exposed amino groups in the collagen network.[8] Therefore, the exposed collagen network may play a role in improved bond strength of AH plus..[6] Root dentine is differentially affected by calcium chelating agents and the proteolytic sodium hypochlorite.[9,10] The type of decalcifying agent has a significant impact on the root dentine wall: EDTA will cause a complete demineralization of the root dentin wall, whilst organic acids cause a gradual demineralization.[11]The latter dentine condition may provide improved resin infiltration.[12] Also, chlorhexidine inhibits matrix metalloproteinases and has a positive effect on dentine bonding.[13]

Recently, an EDTA-based formulation was developed as final rinse solution. QMix (Dentsply Tulsa Dental, Tulsa, OK, USA) contains EDTA, chlorhexidine and a surfactant agent. This solution exhibits a lower level of toxicity than 17% EDTA,[14] which also has low toxicity and antimicrobial activity associated with the ability to remove the smear layer.[15–18]

Minimal information is available regarding the effect of chemical root dentine conditioning with QMix on sealer adhesion. This study was designed to examine the effect of dentine chemical pretreatment using 3% NaOCl, 17% EDTA, 10% citric acid and QMix on the wettability and push-out bond strength of an epoxy resin root canal sealer to root dentin. The null hypotheses tested were (i) the irrigation protocols had no influence on the wettability measured by dynamic contact angle analyzer (ii) the irrigation protocols had no influence on the sealer–dentine bond strength.

Aim and Objective

AIM AND OBJECTIVE

The aim of the study was:

- To evaluate the effect of various final irrigation regimes on the wettability of AH Plus root canal sealer on intraradicular dentine
- To evaluate the effect of various final irrigation regimes on the push out bond strength of AH Plus root canal sealer on intraradicular dentine

Review of Literature

REVIEW OF LITERATURE

Delivanis et al (1983)[19] examined the survivability of F43 strain of Streptococcus sanguis inside root canals filled with gutta-percha and Procosol Cement. They proved that a fluid tight obturation is essential for prevention of survival of microorganisms in the root canals by using the F43 strain of Streptococcus sanguis. Their results confirmed Grossman's hypothesis that if a canal is completely filled laterally and apically any microorganisms remaining inside will not be capable of surviving for a long time.

Drummond et al (1996)[20] evaluated the effect of the following variables on shear dentin-bonding test results: mode of testing (cyclic fatigue versus static loading), surface treatments (32% phosphoric acid, 10% phosphoric acid, and no treatment [unetched], and type of shear test (traditional planar versus push-out). They stated that the bond strength resulting from cyclic fatigue of the etched specimens was approximately 51% of the static loading value. Ten percent phosphoric acid was as effective as 32% phosphoric acid for dentin bonding. They also stated that the push-out test provides a better evaluation of the bonding strength than the conventional shear test because with the push-out test fracture occurs parallel to the dentine-bonding interface, which makes it a true shear test for parallel-sided samples.

Scelza et al (2003)[21]determined the efficacy of 17% EDTA plus 1.25% sodium lauryl ether sulfate (EDTA-T), 10% citric acid, and 17% EDTA with respect to Calcium extraction at 3, 10, and 15 minutes interval using atomic absorption spectroscopy. They showed that EDTA-T extracted the least amount of Calcium from root dentin at the 3 times and that 10% citric acid and 17% EDTA were statistically similar with respect to efficacy.

Goracci et al (2004)[22] compared the trimming and non-trimming variants of the microtensile technique with the micro push-out test in the ability to measure accurately the bond strength of fiber posts luted inside root canals. They concluded that the push-out test appears to be more efficient and dependable than both the trimming and non-trimming versions of the micro- tensile technique.

Zehnder et al (2005)[23] evaluated the effect of reducing surface tension in endodontic chelator solutions on their ability to remove calcium from instrumented root canals. Surface tension in these solutions was measured using the Wilhelmy method. Calcium concentration in eluates was measured using atomic absorption spectrometry. They concluded that incorporation of wetting agents resulted in a reduction of surface tension values by approximately 50% in all tested solutions. They also stated that none of the solutions with reduced surface tension chelated more calcium from canals than their pure counterparts

Buzoglu et al (2006)[24] evaluated ex vivo the effects of combined and single use of EDTA, RC-Prep and NaOCl on the surface free energy of canal wall dentine using the captive bubble technique. They concluded that use of chelating agents alone or in combination with NaOCl decreased the wettability of root canal wall dentine.

Jainaen et al (2007)[25] evaluated the push-out bond strength of the dentine–sealer interface with and without main cone for three resin sealers universal testing machine . They stated that the epoxy resin-based sealer (AH Plus) had the highest push-out bond strength compared with UDMA-based sealers (EndoREZ) and methacrylate resin-based sealer (Resilon) when used with a main cone and sealer. The bond strengths after filling with sealer alone were higher than those with main cone and sealer, and may reflect different patterns of behavior when the sealer is present as a thin layer.

Teixeira et al (2009)[26] compared the shear bond strength (SBS) test and push-out test for evaluation of the adhesion of an epoxy-based endodontic sealer (AH Plus) to dentin and gutta- percha, and to assess the failure modes on the debonded surfaces by means of scanning electron microscopy (SEM). They stated that SEM analysis showed a predominance of adhesive and mixed failures of AH Plus sealer. They also stated that the comparison of the employed methodologies showed that the SBS test produced significantly lower bond strength values than the push-out test, was skillful in determining the adhesion of AH Plus sealer to dentin and gutta-percha, and required specimens that could be easily prepared for SEM, presenting as a viable alternative for further experiments.

Balguerie et al(2011)[27] assessed the tubular adaptation and penetration depth and the adaptation to the root canal walls in the apical, middle, and coronal third of the root canal of 5 different sealers used in combination with softened gutta-percha cones using scanning electron microscopy. The sealers used were Calcium hydroxide epoxy resin (Acroseal), Zinc oxide eugenol (Endobtur), Glass ionomer (Ketac-Endo), Epoxy resin (AH Plus), Silicon (RSA). They stated that AH Plus showed the most optimal tubular penetration and adaptation to the root canal wall of the sealers tested.

De-Deus et al (2012)[28] determined the correlation between leakage and sealer penetration into dentinal tubule using glucose leakage measurements and Confocal Laser Scanning Microscope respectively. They concluded that there was no significant correlation between leakage and sealer penetration into dentinal tubules. The lack of correlation reported is of relevance as sealer penetration into dentinal tubules has been used as an advantageous property during the launch of new root filling materials and techniques.

Silva et al (2012)[29] evaluated the efficacy of smear layer removal of chitosan compared with different chelating agents, and quantified, by atomic absorption spectrophotometry with flame (AASF), the concentration of calcium ions in these solutions after irrigation. They concluded that 15% EDTA, 0.2% chitosan and 10% citric acid effectively removed smear layer from the middle and apical thirds of the root canal. They also stated that 15% EDTA and 0.2% chitosan were associated with the greatest effect on root dentine demineralization, followed by 10% citric acid and 1% acetic acid.

Stojicic et al (2012)[15] assessed the efficacy of a novel root canal irrigant, QMiX, against Enterococcus faecalis and mixed plaque bacteria in planktonic phase and biofilms. In addition, its ability to remove smear layer was evaluated using scanning electron microscopy. They stated that QMiX and NaOCl were superior to CHX and MTAD under laboratory conditions in killing E. faecalis and plaque bacteria in planktonic and biofilm culture. Ability to remove smear layer by QMiX was comparable to EDTA.

Tummala et al (2012)[30] compared the wetting behavior of three different root canal sealers namely zinc oxide (ZnOE), AH plus and GuttaFlow on the root canal dentin surface treated with irrigants and their combination using a dynamic contact angle analyzer. The root dentin surfaces were treated with 17% Ethylenediaminetetraaceticacid (EDTA), 3% sodium hypochlorite (NaOCl) and combination of 17% EDTA and 3% NaOCl. They found that the contact angle values for AH Plus sealer were significantly lower when compared to the other two sealer groups.

Vilanova et al (2012)[31] assessed the bond strength of Epiphany and AH Plus sealers to root canal walls after use of several endodontic irrigants by push-out test using a universal testing machine. They concluded that except for 1% NaOCl, the removal of smear layer with the other irrigants increased the bond strength of AH Plus to intracanal dentine. They also stated that the use of 1% NaOCl for 30 min with 17% EDTA as final irrigant for 5 min increased the bond strength of Epiphany.

Aranda-Garcia et al (2013)[16] evaluated the efficacy of QMiX, SmearClear, and 17% EDTA for the debris and smear layer removal from the root canal and its effects on the push-out bond strength of an epoxy-based sealer by scanning electron microscopy (SEM). They concluded that SmearClear and QMiX are as effective as 17% EDTA in the debris and smear layer removal of the dentinal root surface. The final rinse with these solutions promoted similar push-out bond strength values.

Ballal et al (2013)[32] evaluated the wettability of AH Plus and ThermaSealPlus sealers on intraradicular dentine treated with different irrigating solutions. They stated that when used as a final irrigant, QMix favors the wetting of root canal dentine by both AH Plus and ThermaSeal Plus sealers. Maleic acid shows a promising result when compared to EDTA and NaOCl and wettability of both sealers is the worst on EDTA-irrigated dentine.

Chandrasekhar et al (2013)[14] evaluated the biocompatibility of a new root canal irrigant Q mix™ 2 in 1 in comparison to 0.9% sterile saline, 3% sodium hypochlorite 2% chlorhexidine, and 17% Ethylenediaminetetraacetic acid. They concluded that QMix™ 2 in 1 was shown to be less toxic to the rat subcutaneous tissue than 3% NaOCl, 2% CHX, and 17% EDTA.

López et al (2013)[33] evaluated the dentine wettability of different endodontic irrigation solutions by measuring the contact angle. The dentine segments were randomly divided into six groups according to the irrigating solution: distilled water, 1% sodium hypochlorite, 1% citric acid, 17% EDTA, 1% citric acid + 1% sodium hypochlorite, 17% EDTA + 1% sodium hypochlorite. Then a 3 µl drop of distilled water was laid on each dentine surface with a micro-syringe and images were immediately captured with a super steady shot camera and digitalized. They found that the contact angles were significantly reduced after pre-treatment of the dentine samples with 17% EDTA or 1% citric acid, either alone or in combination with 1% sodium hypochlorite.

AlKahtani et al (2014)[34] evaluated and compared the cytotoxicity of QMix™ root canal irrigating solution on immortalized human bone marrow mesenchymal stem cells (hTERT-MSC-C1) and compared it with that of sodium hypochlorite (NaOCl). They concluded that both the NaOCl and QMix™ solutions are toxic to human bone marrow MSCs. The QMix™ solution, which induces slow cell death, seems to be more biocompatible than the NaOCl solution.

Bohn &Ilie (2014)[35] evaluated the wetting behaviour of three different classes of endodontic sealers, silicone (Roeko- seal Automix), epoxy-resin (2Seal, AH Plus) and methacrylate-based sealers (EndoRez, RealSeal, Real- Seal SE, Seal 3D) on dentine specimens with and without chemical pre-treatment using a contact angle measuring device in a dynamic mode. Half of the discs were rinsed with distilled water, and the remainders were treated with 3% NaOCl, followed by 17% EDTA and 2% CHX to simulate the final rinse under clinical-like conditions. They stated that silicone-based sealer Roekoseal Automix had better wettability than epoxy-resin or methacrylate-based sealers. The irrigation regime significantly favored the wettability of 2Seal and Real- Seal SE.

Das et al (2014)[36] compared the changes in microhardness of root dentin caused by conventional, 6% Morinda Citrifolia Juice (MCJ), and Q Mix irrigation regimens using a Vickers hardness tester. They concluded that the use of QMix regimen will not hamper the root dentin microhardness when compared with MCJ and conventional irrigation regimens.

Elnaghy (2014)[37] investigated the effect of QMix irrigant on the bond strength of glass fibre posts to root dentine and on smear layer removal after post space preparation compared with 5.25% sodium hypochlorite, 2% chlorhexidine digluconate, 17% Ethylenediaminetetraacetic acid (EDTA), 17% EDTA followed by 2% CHX. He concluded that QMix is an effective irrigant that can remove smear layer, open dentinal tubules and simplify the irrigation protocol, without compromising the bonding strength of glass fibre posts cemented with a self-adhesive resin cement to root dentine.

Taneja et al (2014)[38] assessed the effect of QMix, peracetic acid and Ethylenediaminetetraacetic acid on calcium loss and microhardness of root dentine. The calcium loss of the samples was evaluated using the Atomic Absorption Spectrophotometer followed by determination of their microhardness using Vickers Hardness Tester. They stated that Irrigation with NaOCl + 2.25% PAA caused the maximum calcium loss from root dentin and reduced microhardness. A negative correlation existed between the calcium loss and reduction in the microhardness of root dentin.

Arslan et al (2015)[39] compared chlorhexidine and QMix in terms of orange-brown precipitate generation in root canals using a stereomicroscope and also to analyzed the precipitate produced by mixing chlorhexidine and QMix with NaOCl in two separate flasks to determine whether para-chloroaniline was produced. They concluded that Chlorhexidine had significantly higher scores than QMix in terms of orange-brown precipitate formed in the root canals. According to the 1H NMR spectra, para-chloroaniline was present in the mixture of chlorhexidine and NaOCl.

However, the mixture of QMix and NaOCl did not result in para-chloroaniline formation.

Elakanti et al (2015)[40] compared the antimicrobial efficacy of QMix2 in 1, sodium hypochlorite (NaOCl), and chlorhexidine (CHX) against Enterococcus faecalis and Candida albicans by culturing method and colony forming units were counted. They concluded that QMix 2 in 1 demonstrated significant antimicrobial efficacy against Enterococcus faecalis and Candida albicans.

Jardine et al (2015)[41] compared the effect of QMix, BioPure MTAD, 17 % EDTA, and saline on the penetrability of a resin-based sealer AH plus into dentinal tubules using a confocal laser scanning microscope. They concluded that seventeen percent EDTA and QMix promoted sealer penetration superior to that achieved by BioPure MTAD and saline.

Mohan &Pai (2015)[42] assessed the influence of two irrigation regimens having Ethylenediaminetetraacetic acid (EDTA) and Ethylenediaminetetraacetic acid with cetrimide (EDTAC) as final irrigants, respectively, on the dentine wettability for AH Plus sealer by comparing its contact angle formed to the irrigated dentine using a dynamic contact angle analyzer. They concluded that EDTAC as a final irrigant facilitated better dentin wettability than EDTA for AH Plus to promote its better flow and adhesion.

Neelakantan et al (2015)[43]analyzed the influence of irrigation on the chemical interaction between root canal sealers and dentin using Fourier transform infrared spectroscopy (FTIRS) and push-out bond strength test. The root canal sealers tested include, epoxy resin (AH Plus); silicone (RoekoSeal); calcium hydroxide (Sealapex). They found that bond strength of sealers is differentially affected by the irrigation protocol. They also stated that the epoxy resin sealer AH Plus chemically bonds to dentinal collagen and this interaction is influenced by the irrigation protocols.

Uzunoglu et al (2015)[44] evaluated effect of temperatures of QMix and EDTA on the bond-strength of AH Plus. They stated that temperature of the final irrigant does affect the bond strength values of AH plus to root dentin irrigated with EDTA. Bond strength of AH Plus sealer to root canal dentin may improve with QMix.

Balić et al (2016)[45] assessed the antibacterial efficacy of photon-initiated photoacoustic streaming (PIPS) using an Er:YAG laser and sonic-activated irrigation combined with QMiX irrigant or sodium hypochlorite against Enterococcus faecalis intracanal biofilm by culturing and colony forming unit were counted. They concluded that there was no difference in the bacteria reduction between the sonically and laser- activated irrigation, regardless of the irrigant used. Although the activation of the QMiX and NaOCl by the Er:YAG laser did not improve their antimicrobial action, the fact that it generated the greatest number of sterile samples warrants further investigation.

Souza et al (2016)[46] investigated, in vitro, the retaining of 2 % CHX gel, 2 % CHX liquid and QMix within a root canal for 24 h, 30, 90, and 120 days by chemical analysis. They stated that CHX gel, CHX liquid, and QMix were retained in dentin up to 120 days. Significantly less substantivity was observed for QMix, irrespective of the period of time. They also stated that no differences were noted between CHX gel and CHX liquid after 30, 90, and 120 days of evaluation.

Azar et al (2000)[47] studiedthe cytotoxic effects of a new epoxy resin-based root canal sealer (AH-plus), and compared with AH26 and zinc oxide-eugenol sealers, in vitro on a culture of human gingival fibroblasts. They concluded that cytotoxicity of the AH-plus was confined to the early period of experiment at 1 and 4 hour intervals and was no longer detectable after 4 hr of mixing. They also stated that that AH plus is more biocompatible than the other two sealers, making it suitable for endodontic practice.

Dogan et al (2001)[48]evaluated the effect of combined and single use of EDTA, RC-Prep, and NaOCl on mineral content of root dentin using scanning electron microscope (SEM) and energy dispersion spectrometric microanalysis. They concluded that the use of NaOCl irrigation as final flush altered the effectiveness of chelating agents on root dentin surface. They also stated that the use of EDTA and RC-Prep alone did not change the mineral content of root dentin significantly.

Khedmat et al (2008)[49] compared the efficacy of three chelating agents in smear layer removal using scanning electron microscopy (SEM). They stated that the application of 1 mL of SmearClear, 17% EDTA, and 10% citric acid for 1 minute followed by 3 mL of 5.25%NaOCl was not sufficient to completely remove the smear layer, especially in the apical third. They also stated that the addition of surfactants to EDTA in SmearClear did not result in better smear layer removal compared with EDTA alone.

Hu et al (2010)[50] studied the effects of irrigation solutions on dentin wettability and roughness by using contact angle measurements and AFM analysis respectively. The irrigants used were 17% EDTA, 5.25% NaOCl and 3% H_2O_2. They stated that all the irrigation solutions in this study influenced the physicochemical properties of dentin surfaces, such as wettability and roughness. Hence, the effects of the changes of physicochemical properties on the bacterial adhesion and restorative adhesive procedure should be further studied. They also stated that due consideration should be given to these effects as the irrigants are used during root canal treatment.

Dai et al (2011)[51] examined the ability of two versions of QMix, an experimental antimicrobial irrigant, on removal of canal wall smear layers and debris using an open canal design. The two versions tested were: QMix solution I with pH equal to 8 and QMix solution II with pH equal to 7.5. They stated that the two versions of the experimental antimicrobial (QMix) are as effective as 17% EDTA in removing canal wall smear layers from the entire root canal space in straight root canals after the initial rinse of NaOCl. They also stated that similar to BioPure MTAD and EDTA, these two QMix versions are ineffective in clearing debris completely from the canal spaces when the corresponding irrigant is delivered via the insertion of a side-vented needle to 1 mm above the apical seat.

Neelakantan et al (2011)[52] evaluated the impact of dentine conditioning on sealing ability and dentine bond strength of AH plus by fluid transport model and push out tests respectively. The irrigants used were 3% NaOCl, 17% EDTA, 7% maleic acid (MA) or 2% chlorhexidine. They concluded that a final flush with a decalcifying agent appears advisable, whilst a final flush with NaOCl caused untoward effects on bond strength. They also stated that conditioning of canal walls by maleic acid, resulted in superior sealing ability and higher epoxy resin bond strength compared to EDTA. The two outcomes investigated, fluid transport and dentine bond strength, were strongly negatively correlated to each other.

Stelzer et al (2014)[53] evaluated the influence of different endodontic irrigants namely sodium hypochlorite, chlorhexidine, and EDTA on the push-out bond strength of RealSeal SE (SybronEndo, Orange, CA) and AH Plus using the universal testing machine. They stated that the push-out bond strength of the conventional AH Plus sealer is usually higher than that of RealSeal SE. They also stated that the bond strength of RealSeal SE is highly influenced by the irrigant used. Within the AH Plus groups, no significant differences existed in bond strength for different irrigation protocols.

Gründling et al (2015)[54] determined the effect of QMix, 17% EDTA, 2% chlorhexidine solution, and 3% Sodium hypochlorite in reducing the endotoxin content in root canals by lipopolysaccharide (LPS) quantification using Limulus Amebocyte Lysate(LAL) assay. They concluded that the chemical action of NaOCl, Chlorhexidne and EDTA has not been able to reduce LPS load inside the root canal system whereas QMix seemed to reduce the LPS load when compared to the other irrigants.

Kaushik et al (2015)[55]evaluated the contact angle between epoxy resin sealer and dentin treated with different irrigant solutions using Rame Hart Goniometer. The irrigants used were NaOCl, QMix and 0.1% octenidine hydrochloride. Comparative evaluation of wettability was done before and after removal of smear layer. They stated that QMix irrigated samples showed least values of contact angle, and thus maximum wettability of the sealer followed by 0.1% octenidine hydrochloride followed by 3% NaOCl. They also stated that the removal of smear layer using EDTA further reduced contact angle of AH Plus in samples treated with 0.1% octenidine hydrochloride.

Tuncel et al (2015)[56] evaluated the effect of various chelating solutions on the radicular push-out bond strength of calcium silicate-based and resin-based root canal sealers using a universal testing machine. The chelating solutions being tested were 17% EDTA; 9% etidronic acid; 1% peracetic acid. The sealers used were an epoxy resin-based sealer (AH Plus) and a calcium silicate-based sealer (iRoot SP). They concluded that the tested chelating solutions did not improve the bond strength of AH Plus and iRoot SP to the radicular dentin.

Materials and Methods

MATERIALS AND METHODS

ARMAMENTARIUM

INSTRUMENTS USED:

1. Diamond disc with mandrel
2. 600 Grit silicon carbide paper
3. Scale
4. Endomotor (Endo-Mate DT, NSK Nakanishi International, Japan)
5. Protaper rotary files (Dentsply Maillefer, Switzerland)
6. K files (Mani,Tochigi, Japan)
7. Endoblock (Dentsply Maillefer, Switzerland)
8. Stainless steel cement spatula
9. Glass slab
10. 5ml syringe (Dispo Van)
11. Spirit lamp
12. Glass slab
13. Tweezer
14. Lentulo spiral
15. Guttapercha condenser
16. Spreaders
17. Humidity chamber
18. Hard tissue microtome (Leica, Germany)
19. Autoclave (Unique clave C-79, Confident)
20. Dynamic contact angle analyzer (GBX Digidrop)
21. Universal testing machine (ZwickRoell Z010)

MATERIALS USED:

1. 3% Sodium hypochlorite (Vensons India, Bengaluru)
2. Saline (Aculife Healthcare, India)
3. 10% Citric acid (Chenchems, Chennai)
4. 17% EDTA (Chenchems, Chennai)
5. Qmix (Dentsply Tulsa Oklahoma)
6. AH Plus sealer (Dentsply DeTrey GmbH, Konstanz, Germany)
7. Guttapercha (Dentsply Maillefer)
8. Paper points (Dentsply Maillefer)

SOURCE OF SAMPLES:

One hundred freshly extracted human single rooted mandibular premolar teeth that were extracted for periodontal and orthodontic reasons were collected from the Department of Oral and Maxillofacial Surgery, Sri Ramakrishna Dental College and Hospital, Coimbatore.

INFECTION CONTROL PROTOCOL FOR THE TEETH COLLECTED FOR THE STUDY:

Collection, storage, sterilization and handling of extracted teeth were followed according to the Centre for Disease Control and Prevention guidelines.[57,58]

(CDC) recommendations and guidelines as follows:

1. Handling of teeth was always done using gloves, mask and protective eyewear
2. Teeth were cleaned of visible blood and gross debris
3. Teeth were maintained in a hydrated state in a well-constructed closed container containing distilled water during transport
4. Teeth were immersed in 10% formalin for 7 days, following which the liquid was discarded and the teeth were transferred into separate jars containing distilled water
5. The initial collection jars, lids and the gloves employed were discarded into biohazard waste receptacles
6. As and when required, teeth were removed from the jars with cotton pliers

INCLUSION CRITERIA:

1. Intact, single rooted lower premolars with single root canal
2. Teeth with complete root formation
3. Teeth with patent canals
4. Teeth without anatomic variations
5. Teeth free of dental caries and root canal fillings.

EXCLUSION CRITERIA

1. Teeth with open apices
2. Calcified canals
3. Multi rooted teeth

4. Variations in radicular anatomy
5. Internal or external root resorption
6. Teeth with fractured roots

PROCEDURE:

ASSESSMENT OF DENTIN WETTABILITY OF AH PLUS ROOT CANAL SEALER FOLLOWING DIFFERENT IRRIGATION REGIMENS:

TOOTH SAMPLE PREPARATION:

To reveal root dentine, 20 premolars were cross-sectioned into two separate 1-mm-thick dentine discs per tooth with a diamond disc used under distilled running water. (Figure 2) 40 dentin discs were thus obtained. To create uniform flat surfaces, the dentine discs were subsequently ground with 600-grit silicon carbide paper under distilled water to remove any surface scratches and to provide a smooth surface for the analysis.

SAMPLE TREATMENT:

Dentin discs were then divided randomly into 4 groups (n = 10) depending on the irrigation regimen:

- Group I: 5 ml of 3% NaOCl.
- Group II: 5 ml of 3% NaOCl + 5 ml of 17% EDTA.
- Group III: 5 ml of 3% NaOCl + 5 ml of 10% citric acid.
- Group IV: 5 ml of 3% NaOCl + 5 ml of Q mix.

Irrigation was carried out for one minute. Five milliliters saline was used between the irrigants in groups 2 -4. Following irrigation, all the samples were rinsed with 5 ml of saline and dried with paper points.

CONTACT ANGLE MEASUREMENT

The contact angle was measured using a Dynamic Contact Angle Analyzer (GBX Digidrop, IIT Chennai) under standard conditions of temperature and relative humidity. (Figure 9) This equipment is used for measuring both the static and dynamic contact angles. AH Plus (Dentsply DeTrey, Konstanz, Germany), an epoxy resin-based sealer, was mixed according to the manufacturer's instructions. (Figure8)

Each sample was positioned on a flat glass surface of the Dynamic Contact Angle Analyzer, and a controlled volume droplet (0.1 ml) of the sealer was placed on the dentinal surface of the sample. (Figure 10) The volume of the sealer was controlled manually using a syringe and calculated by using a Digidrop software. Ten drops of the same sealer was evaluated for each treatment (n = 10) and the spreading process was recorded for 60 seconds. Images of the droplets were then captured using the Digidrop software to determine the static contact angles made by the sealers on root canal dentine.

ASSESSMENT OF PUSH OUT BOND STRENGTH OF AH PLUS ROOT CANAL SEALER FOLLOWING DIFFERENT IRRIGATION REGIMENS:

TOOTH SAMPLE PREPARATION:

The teeth were decoronated at the cementoenamel junction by using a diamond disk under distilled running water. (Figure 1) The root lengths were standardized to 12 mm and radiographed at 2 angulations to confirm the presence of a single canal. A size 10 K file was placed in the canal until it was visible at the apical foramen. The working length was determined by subtracting 1mm from this measurement. Biomechanical preparation was performed with ProTaper Universal NiTi rotary instrument. (Figure 11 &12) Canals were enlarged up to F4 at the working length. The irrigating solution was delivered via a sterile 30-gauge single side vented needle (Ammdent Canal clean) which penetrated 2mm short of the working length.

The samples were divided into 4 groups (n = 20) according to the final irrigation regimen.

- Group I: 5 ml of 3% NaOCl
- Group II: 5 ml of 3% NaOCl + 5 ml of 17% EDTA.
- Group III: 5 ml of 3% NaOCl + 5 ml of 10% citric acid.
- Group IV: 5 ml of 3% NaOCl + 5 ml of Q mix.

Sodium hypochlorite was used during instrumentation. Final irrigant was allowed to remain in the canal for 1 minute. 5ml saline was used between the irrigants in groups 2 -4. All groups received a final flush with 5 ml of saline and were then dried with absorbent paper points. AH Plus sealer (Dentsply DeTrey GmbH, Konstanz, Germany) was mixed according to the manufacturer’s instructions and was coated on the root canal walls using a letulospiral. Obturation was done with F4 size master cone by cold lateral compaction. The teeth were radiographed at 2 angulations. Samples with voids or bubbles were discarded. Specimens were placed in 100% humidity for 48 hours to ensure complete setting of the sealer. (Figure 13)

After this period, the specimens were embedded in self cure acrylic resin (Figure 14& 15) and each root was transversely sectioned using a hard tissue microtome (Leica Germany). One mm slices of mid-root dentin were made to achieve a main cone diameter approximately 1mm. (Figure 16 - 18)

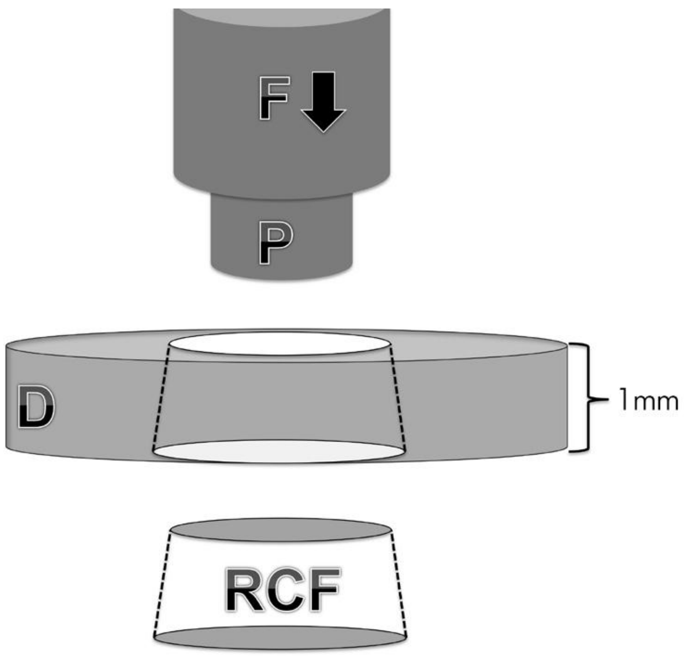
MEASUREMENT OF DISLOCATION RESISTANCE BY PUSH-OUT BOND STRENGTH TEST

Each root section was subjected to a compressive load via a universal testing machine at a crosshead speed of 1 mm/min by using stainless steel plunger 0.7mm in diameter, which provided the most extensive coverage over the filling material, without touching the canal wall. (Figure 19).The plunger was positioned so that it contacted only the filling material. Push-out force was applied in an apico-coronal direction until bond failure occurred, manifested by extrusion of the filling material and a sudden drop in load deflection. The force was recorded by using data analysis software. The maximum failure load was recorded in Newtons, and push-out bond strength was calculated in megapascals (MPa).

Bond strength (MPa)= Load in newton /Area of bonded surface .

The area of bonded surface is given by the formula 2πr*h. π is a constant with an approximate value of 3.14, r is the internal diameter of the root canal and h is the height of the specimen. All the data obtained were recorded, tabulated and statistically evaluated.

SCHEMATIC REPRESENTATION OF THE PUSH-OUT TEST



Direction of force (F); cylindrical plunger (P); root dentin cylinder (1 mm thick) (D); root canal filling (sealer and core material) (RCF).

FIGURE 1: DECORONATED TOOTH SPECIMEN FOR PUSH-OUT BOND STRENGTH TEST

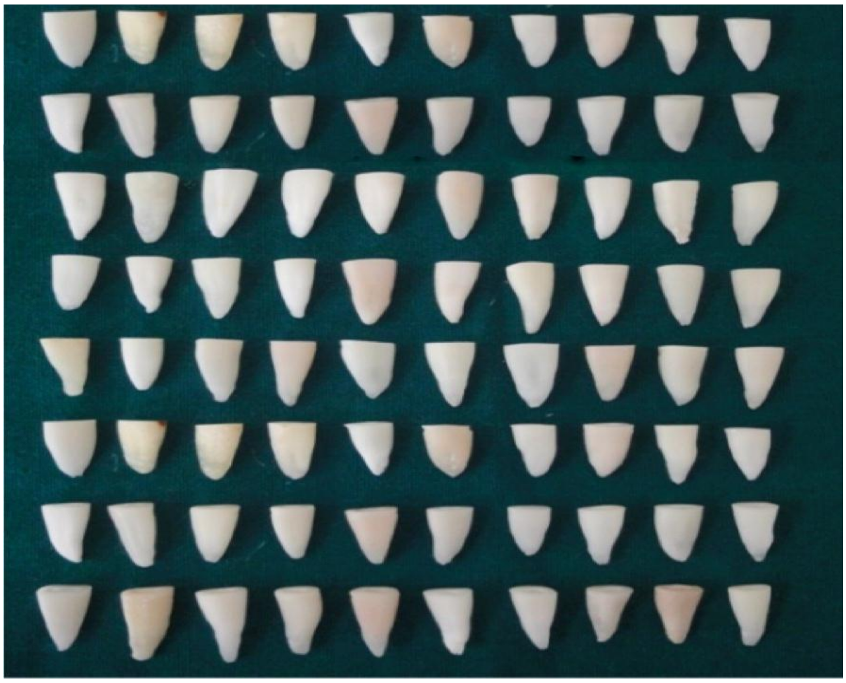


FIGURE 2: DENTIN DISCS FOR CONTACT ANGLE MEASUREMENT

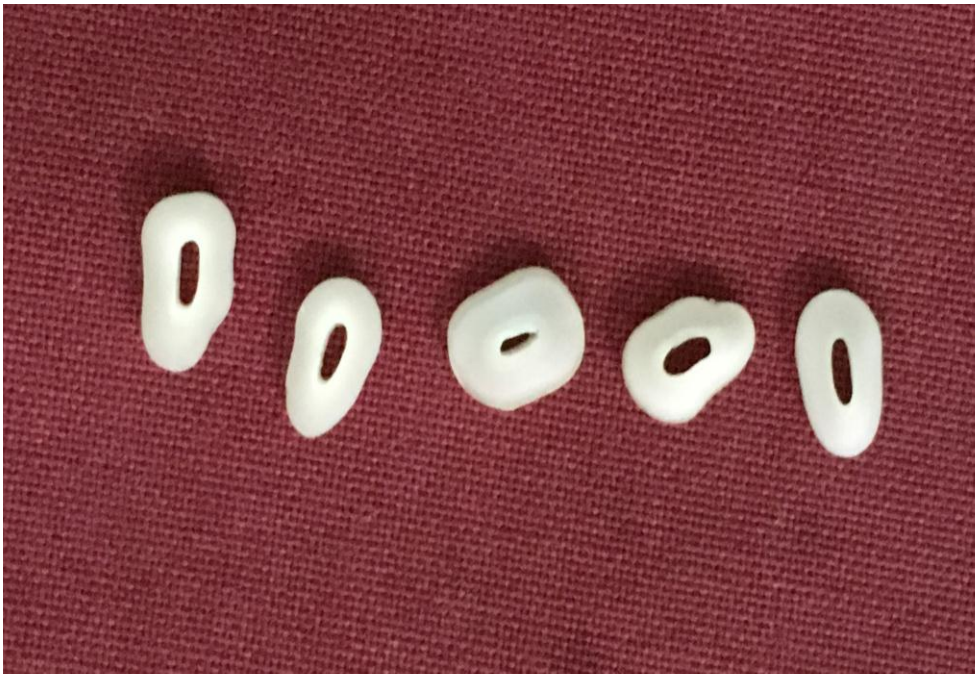


FIGURE 3: ARMAMENTARIUM USED FOR THE STUDY

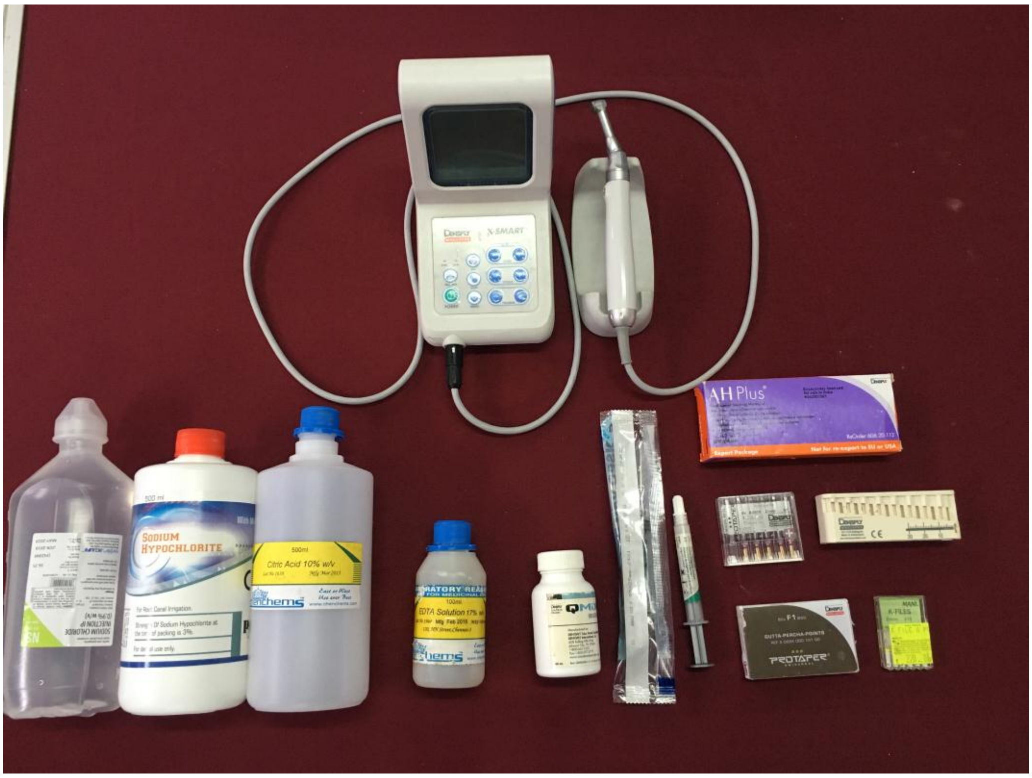


FIGURE 4: 3% SODIUM HYPOCHLORITE

FIGURE 5: 17% EDTA

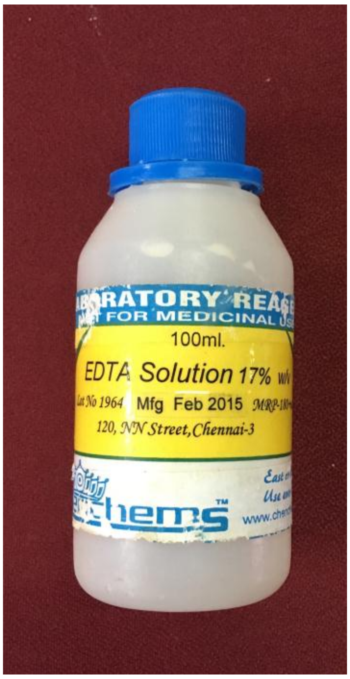


FIGURE 6: 10% CITRIC ACID

FIGURE 7: QMIX

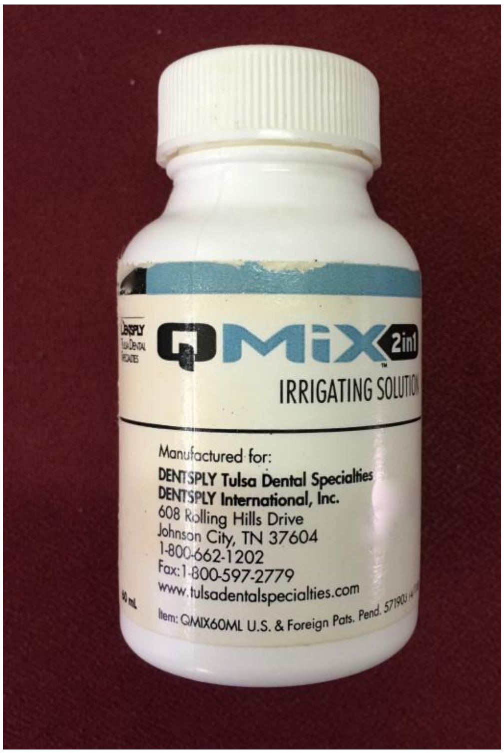


FIGURE 8: AH PLUS

FIGURE 9: DYNAMIC CONTACT ANGLE ANALYSER

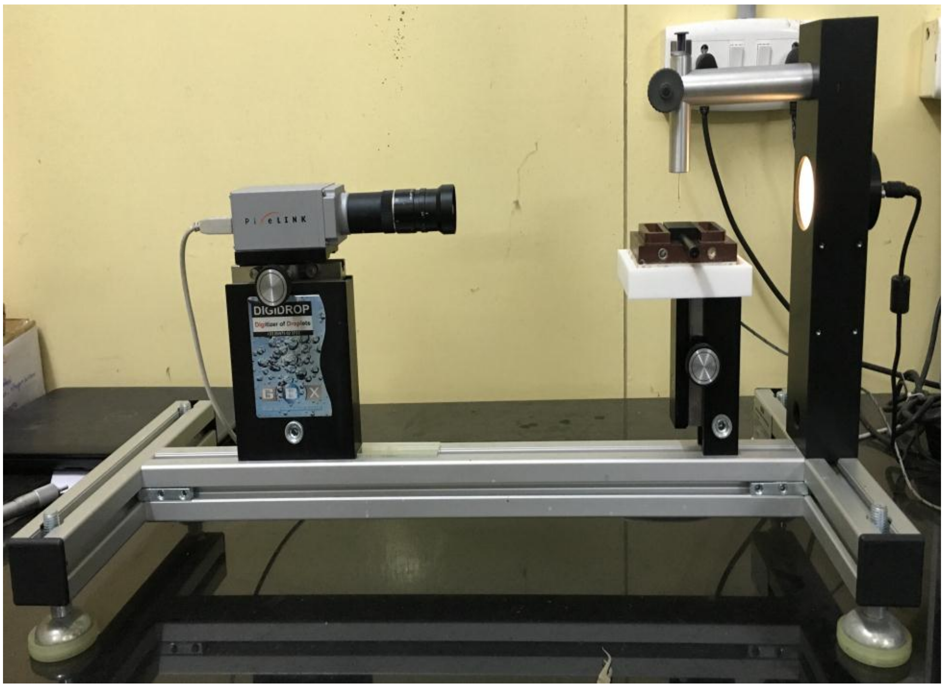


FIGURE 10: EXPERIMENTAL SET UP WITH DENTIN DISC IN POSITION

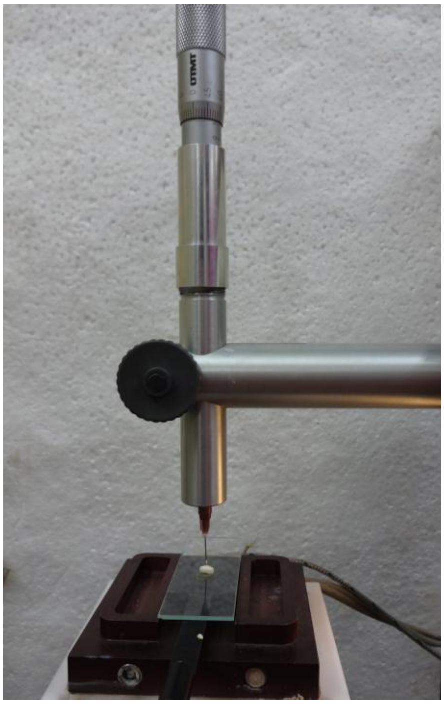


FIGURE 11: BIOMECHANICAL PREPARATION OF DECORONATED SAMPLES

FIGURE 12: PROTAPER ROTARY SYSTEM AND K FILE

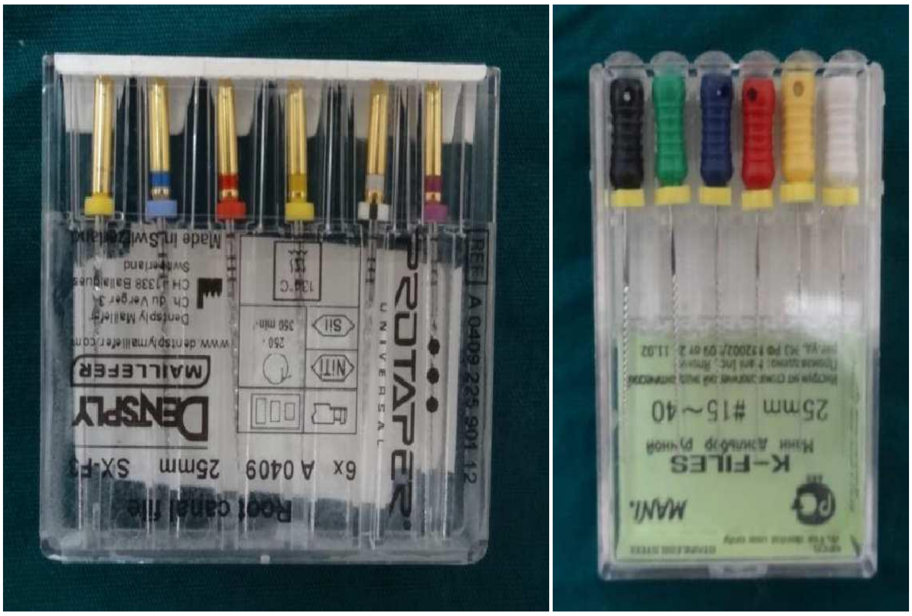


FIGURE 13: TEETH SPECIMEN IN HUMIDITY CHAMBER

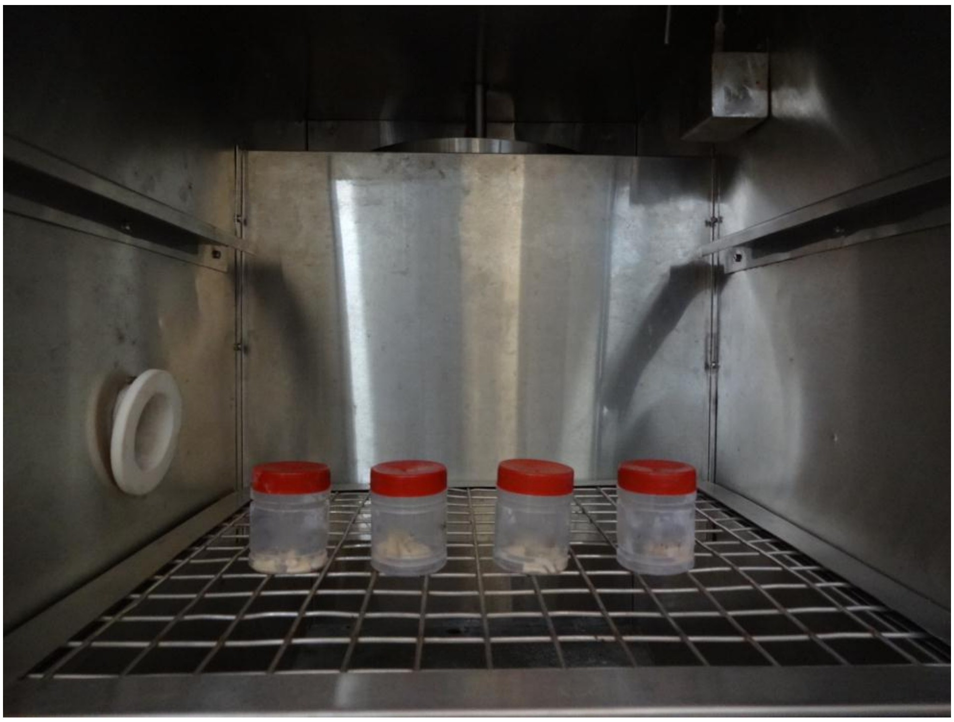


FIGURE 14: ELASTOMERIC IMPRESSION SAMPLES MOLD WITH MOUNTED ROOT

FIGURE 15: TOOTH SAMPLES IN SELF CURE ACRYLIC

FIGURE 16: HARD TISSUE MICROTOME

FIGURE 17: CROSS SECTION AT MIDDLE THIRD DONE WITH A HARD TISSUE MICROTOME

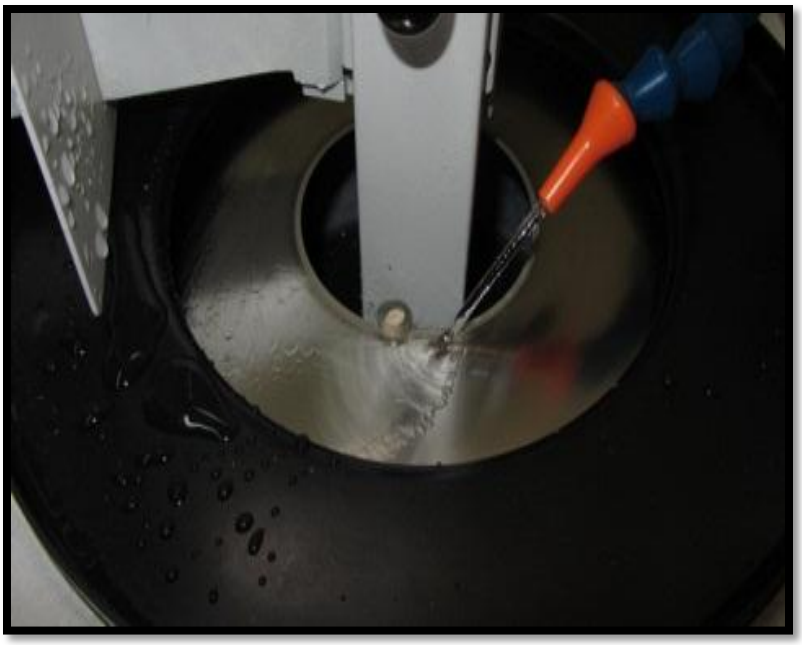
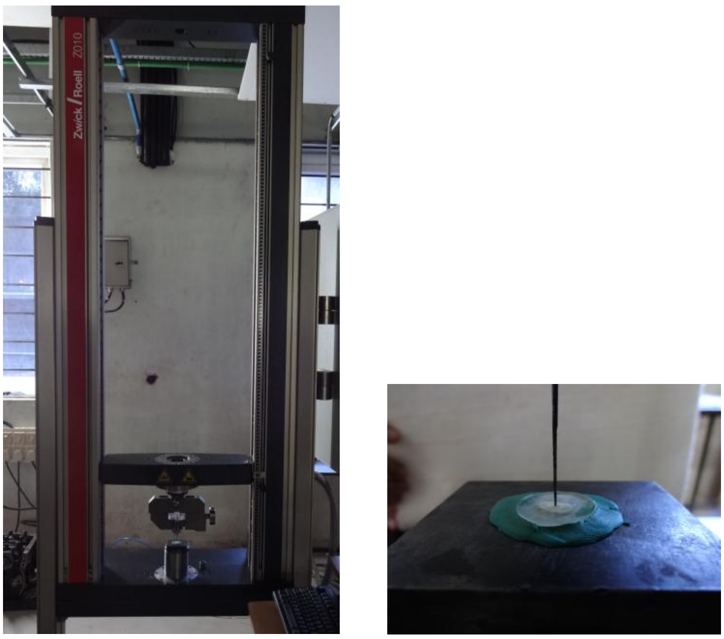


FIGURE 18: MID ROOT DENTIN SLICES

FIGURE 19: EXPERIMENTAL SET UP WITH UNIVERSAL TESTING MACHINE AND THE SAMPLE IN POSITION



STATISTICAL ANALYSIS

Data was analyzed using SPPS VERSION 16 (Statistical Software). The level of significance was set at P < 0.05 and with a confidence interval of 95%. Inter and intra group comparisons were done with one-way ANOVA followed by a pairwise comparison with Turkey’s post hoc test for evaluating the wettability and push out bond strength of compared groups.

Results

RESULTS

STATISTICAL ANALYSIS OF WETTABILITY OF AH PLUS ON ROOT CANAL DENTIN FOLLOWING VARIOUS IRRIGATION REGIMES

DESCRIPTIVES (TABLE 1)

WETTABILITY

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Sodium Hypochlorite	10	48.5000	1.65597	.52366	47.3154	49.6846	46.40	51.10
NaOCl + EDTA	10	61.0800	3.45311	1.09197	58.6098	63.5502	54.30	66.20
NaOCl + Citric Acid	10	60.1600	3.66521	1.15904	57.5381	62.7819	53.10	65.20
NaOCl + Q Mix	10	33.9500	2.92204	.92403	31.8597	36.0403	29.30	38.80
Total	40	50.9225	11.49599	1.81767	47.2459	54.5991	29.30	66.20

ONE WAY ANOVA (TABLE 2)

WETTABILITY					
	Sum of Squares	df	Mean Square	F	**Sig.**
Between Groups	4824.405	3	1608.135	175.569	**.001**
Within Groups	329.745	36	9.160		
Total	5154.150	39			

The mean values and the standard deviations of the wettability of each group are shown in Table 1. ANOVA test concluded that the values were highly statistically significant ($p < 0.05$), where NaOCl + QMix (Group IV) had the lowest contact angle and EDTA (Group II) had the highest contact angle.

MULTIPLE COMPARISONS (TABLE 3)

WETTABILITY

Tukey HSD

(I) Groups	(J) Groups	Mean Difference (I-J)	Std. Error	**Sig.**	95% Confidence Interval	
					Lower Bound	Upper Bound
Sodium Hypochlorite	NaOCl + EDTA	-12.58000*	1.35348	**.000**	-16.2252	-8.9348
	NaOCl + Citric Acid	-11.66000*	1.35348	**.000**	-15.3052	-8.0148
	NaOCl + Q Mix	14.55000*	1.35348	**.000**	10.9048	18.1952
NaOCl +EDTA	Sodium Hypochlorite	12.58000*	1.35348	**.000**	8.9348	16.2252
	NaOCl + Citric Acid	.92000	1.35348	**.904**	-2.7252	4.5652
	NaOCl + Q Mix	27.13000*	1.35348	**.000**	23.4848	30.7752
NaOCl + Citric Acid	Sodium Hypochlorite	11.66000*	1.35348	**.000**	8.0148	15.3052
	NaOCl + EDTA	-.92000	1.35348	**.904**	-4.5652	2.7252
	NaOCl + Q Mix	26.21000*	1.35348	**.000**	22.5648	29.8552
NaOCl + Q Mix	Sodium Hypochlorite	-14.55000*	1.35348	**.000**	-18.1952	-10.9048
	NaOCl + EDTA	-27.13000*	1.35348	**.000**	-30.7752	-23.4848
	NaOCl + Citric Acid	-26.21000*	1.35348	**.000**	-29.8552	-22.5648

*. The mean difference is significant at the 0.05 level.

Table 3 shows the intergroup comparison by Post Hoc Tukey test, where high statistical difference was found when group 1 was individually compared with group 2, group 3 and group 4. There was no statistical difference between group 2 and group 3. Group 4 showed high statistical difference when compared individually with group 3 and group 4.

STATISTICAL ANALYSIS OF PUSH OUT BOND STRENGTH OF AH PLUS ON ROOT CANAL DENTIN FOLLOWING VARIOUS IRRIGATION REGIMES

PUSHOUT RESULTS

DESCRIPTIVES (TABLE 4)

Groups	Number	Mean	Std. Deviation	Minimum	Maximum
SodiumHypochlorite	20	4.892105	.8366107	3.7000	6.7500
NaOCl + EDTA	20	7.151000	1.2995906	4.7900	9.9000
NaOCl+ CitricAcid	20	6.814000	1.3374776	4.3700	9.2300
NaOCl + QMix	20	7.194500	1.4243132	4.4600	9.9800
Total	80	6.533418	1.5456663	3.7000	9.9800

ANOVA FOR PUSH OUT TESTS (TABLE 5)

Groups	Mean	Std. Deviation	df	Mean Square	F	P value
SodiumHypochlorite	4.892105	.8366107	3	23.043	14.743	**.000**
NaOCl + EDTA	7.151000	1.2995906				
NaOCl + CitricAcid	6.814000	1.3374776				
NaOCl + QMix	7.194500	1.4243132				

The mean values and the standard deviations of the push out bond strength of each group are shown in table 4. ANOVA test concluded that the values were highly statistically significant ($p < 0.05$), where NaOCl + QMix group (7.194500 MPa) had the highest push out bond strength and sodium hypochlorite group (4.892105 MPa) had the lowest bond strength.

MULTIPLE COMPARISONS (TABLE 6)

PUSH OUT TESTS

Post hoc analysis (Tukey HSD) to analyse the diffence between the groups

Groups		Mean Differnece	P value
SodiumHypochlorite	NaOCl + *EDTA*	-2.2588947*	***.000***
	NaOCl + *CitricAcid*	-1.9218947*	***.000***
	NaOCl + *QMix*	-2.3023947*	***.000***
NaOCl + EDTA	*SodiumHypochlorite*	2.2588947*	***.000***
	NaOCl + *CitricAcid*	.3370000	.829
	NaOCl + *QMix*	-.0435000	1.000
NaOCl + CitricAcid	*SodiumHypochlorite*	1.9218947*	***.000***
	NaOCl + *EDTA*	-.3370000	.829
	NaOCl + *QMix*	-.3805000	.771
NaOCl + QMix	*SodiumHypochlorite*	2.3023947*	***.000***
	NaOCl + *EDTA*	.0435000	1.000
	NaOCl + *CitricAcid*	***.3805000***	***.771***

*. The mean difference is significant at the 0.05 level.

Table 6 shows the intergroup comparison by Post Hoc Tukey test, where high statistical difference was found when group 1 was individually compared with group 2, group 3 and group 4. There was no statistical difference when group 2 was individually compared with group 3 and group 4.

FIGURE 20: DISTRIBUTION OF GROUPS BASED ON MEAN VALUES.

(WETTABILITY)

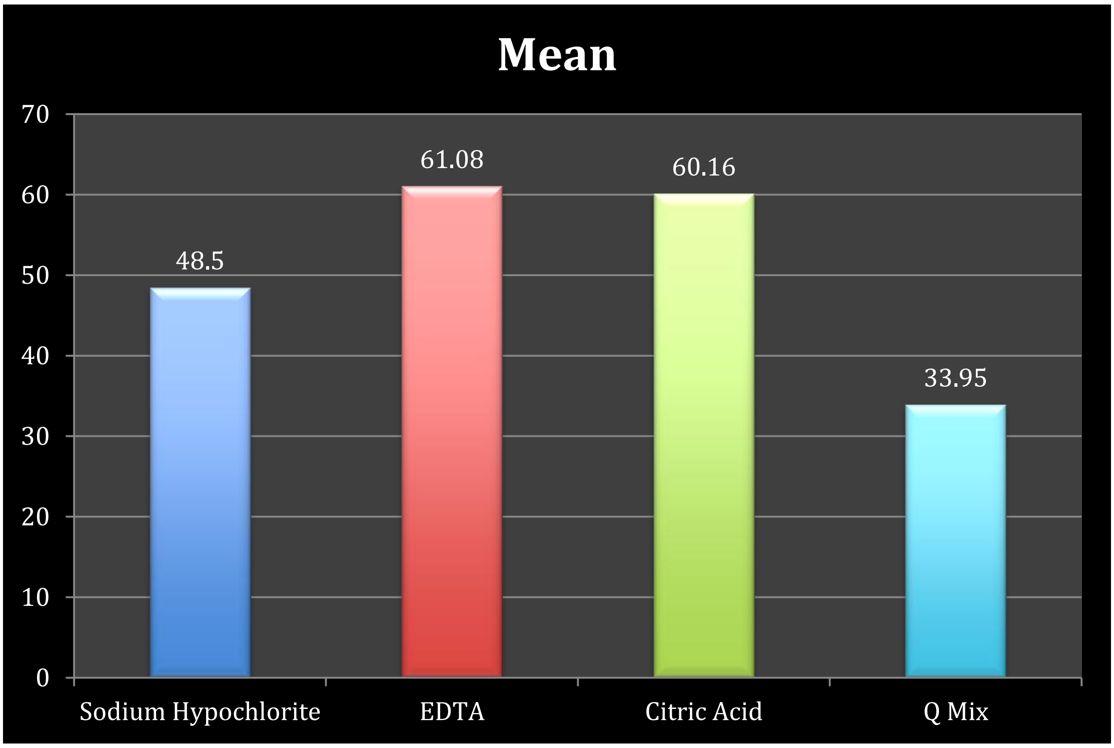
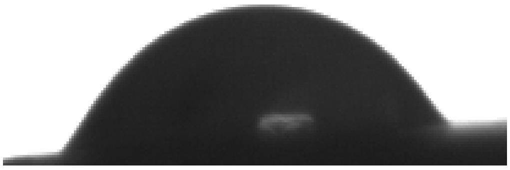


FIGURE 21: REPRESENTATIVE IMAGES OF SESSILE DROPS OF ROOT CANAL SEALERS APPLIED TO ROOT CANAL DENTINE TREATED WITH DIFFERENT IRRIGATING SOLUTIONS.

3 %SODIUM HYPOCHLORITE

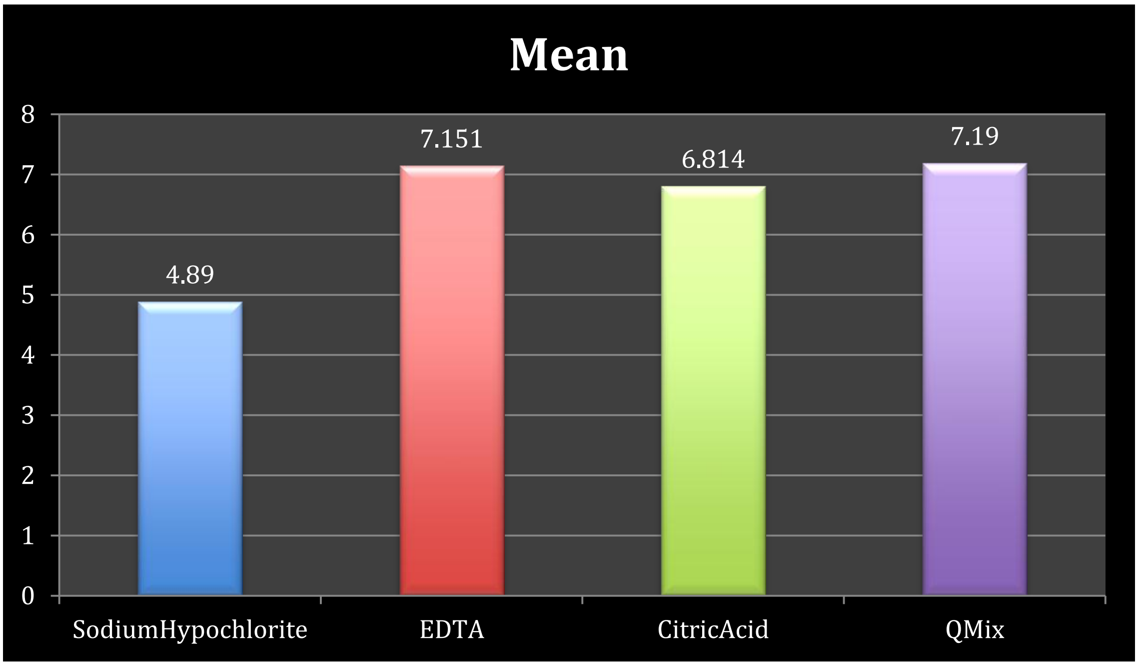


17% EDTA

10% CITRIC ACID

QMIX

FIGURE 22: DISTRIBUTION OF THE GROUPS BASED ON MEAN VALUES (PUSH OUT BOND STRENGTH)



Discussion

DISCUSSION

Attaining a fluid tight seal in all dimensions, within the entire root canal system, is one of the key factors contributing to the long term success of root canal treatment.[59] This may be achieved by three-dimensional obturation of the root canal system to prevent oral and apical microleakage. In the context of canal filling, endodontic sealers play an important role as they fill root canal wall irregularities, accessory canals, ramifications and the space between guttapercha and canal walls.[60]

Optimum adhesion requires intimate contact between the sealer and the substrate to facilitate molecular attraction and allow either chemical adhesion or penetration for micromechanical surface interlocking. The adhesion processes are mainly influenced by the relative surface free energy (wetting ability) of the solid surface.[60,61] Wetting is the property of a liquid to sustain contact with a solid surface, depending on the relative surface energies of the solid and the liquid. [63-65]Adhesion of a root canal sealer depends on the wetting ability of the sealer.[66]

Several studies showed the adhesion and bond strength of the obturation systems was found to be differently affected by the various irrigation regimens applied. [16,67,68] Dentin surface treatment with different irrigation regimens may cause alteration in the chemical and structural composition of human dentin, thereby changing its permeability and solubility characteristics[69,48] and hence affecting the adhesion of materials to dentin surfaces.[61]

The utilization of sodium hypochlorite (NaOCl) irrigant in endodontics is justified by its undeniable importance as a result of both its wide-spectrum antimicrobial activity and its properties as an organic tissue solvent. However, it is known to be highly irritant to the periapical tissues, mostly at high concentrations and it is unable to remove the smear layer by itself, as it dissolves only organic material .[70-72] Chlorhexidine digluconate (CHX) is a potent antiseptic with a broad-spectrum antimicrobial activity and low grade of toxicity; however, it has no tissue-dissolving capability, and consequently, it cannot replace NaOCl.[71-73] Ethylenediaminetetraacetic acid (EDTA) is effective at dissolving inorganic material, including hydroxyapatite; on the other hand, it has little or no effect on organic tissue and it does not possess antibacterial activity.[72] Thus most of the commonly used irrigants have only few desirable properties.

By virtue of the known drawbacks of all endodontic irrigants, developing improved irrigating solutions for endodontics remains an area of great interest.[48] Recently, QMix (Dentsply Tulsa Dental, Tulsa, OK, USA), a novel irrigant for smear layer removal with added antimicrobial agents, has been developed. It contains EDTA, CHX and a detergent. QMix is a clear solution ready to use with no chair- side mixing.[15,17] It is effective against bacterial biofilms and removes smear layer equivalent to 17% Ethylenediaminetetraacetic acid (EDTA)[15,17]

AH plus is a epoxy resin based endodontic sealer which can be used with guttapercha to obtain a three dimensional filling.[74] AH Plus has better penetration into the micro-irregularities because of its creep capacity and long setting time, which increases the mechanical interlocking between sealer and root dentin.[47] Due to its properties, such as low solubility, minimal expansion, adhesion to dentin, and very good sealing ability, AH Plus is considered as a benchmark "Gold Standard." [74]

It would be clinically relevant to know whether an irrigation regimen having QMIX as final irrigant would have any effect on dentine wettability and adhesion of root canal sealers when compared to the conventional irrigants namely sodium hypochlorite, EDTA and citric acid.

The tendency of a liquid to spread on a solid surface is expressed in the formation of a contact angle between the surface of the fluid and the surface of the solid.[63-65] Thus, the contact angle provides an inverse measure of wettability[75.76], meaning that the lower the contact angle, the better the wettability.[63,75,76] Therefore, the measurement of contact angles can help indirectly assess the sealing ability of endodontic sealers.

Also adhesion of root canal sealers can be assessed by push-out test as the test allows an accurate standardization of the specimens,[1,2] while the micro-push- out test, for use in smaller areas, yields the development of a more uniform shear strength without the interference of the tensile component, thus producing a stress more reliably directed at the adhesive interface.[22,77]

The goal of the current research was two fold: First, to analyze the effect of various irrigants (sodium hypochlorite, EDTA, citric acid and QMix) on wettability of epoxy resin based root canal sealer - AH plus; Secondly, to analyze the effect of various irrigants on the push out bond strength of AH plus.

The contact angle can be measured using a captive bubble or a sessile drop technique. In the present study, the sessile drop method was used. With this approach, the contact angles of a liquid drop on flat surfaces can be maintained in a dry environment. The state of hydration of the dentin surface is also shown to affect the contact angle.[78] Although in most studies the solid surfaces were dried with air,[78,79] in the present study the samples were dried with paper points to simulate conditions in the root canal system.

The factors affecting the contact angle on the liquid side include the surface tension, and those on the solid side include the hydrophobic effect caused by changes in the porosity as a result of surface roughness, and the surface energy effect based on the changes in the 3-dimensional structure of the surface molecules.[80] Polishing the dentine surface was done to reduce the influence of roughness on the surface energy of the root dentine wall and thus to reduce its influence on the contact angle measurement. [81]

All static contact angle measurements were performed by using a controlled-volume (0.1 ml) of the sealer using a Digidrop software to measure the volume. This was done to nullify the effect of volume change on the value of contact angle measurement.[82]

The results of the present study demonstrated that, EDTA increased the contact angle of AH Plus sealers when compared to the other tested irrigants. Hence, wettability or spreading of both sealers on root canal dentine was reduced when EDTA was used as the final irrigant. This finding is in agreement with the results reported in previous studies.[24,32,81] This occurs because dentine consists predominantly of two different substrates: collagen, which has a low surface free energy, and carbonated apatite, which has a high surface energy.[83] Due to the demineralizing ability of EDTA and its absence of a surfactant effect, the use of EDTA as the final canal irrigant results in a thin layer of demineralized collagen fibrils on the dentine surface.[84] In the absence of an accompanying surfactant, the presence of this layer of collagen fibrils is responsible for the poor wettability of root canal sealers on the EDTA-irrigated dentine.[85]

Citric acid produced similar results as EDTA. This may be due to the similar decalcifying effect of EDTA and citric acid[21] and also the lack of surfactant.

Contrary to the findings of this study **López et al in 2013** discovered that 1% citric acid and 17% EDTA, alone or in combination with sodium hypochlorite, increased the surface free energy of dentine.[33] However they measured water contact angles whereas in our study the contact angle of the root canal sealer was measured. **Tummala et al in 2012** reported that wettability of AH plus sealer was better when root dentin was treated with EDTA compared to NaOCl alone. This may be explained by the difference in the irrigation time (5 minutes) and the time of contact angle measurement (30 minutes after the droplet was placed).[30]

From the results of the present study we infer that, QMix is shown to have the best wetting ability on root canal dentine surface when compared to the other irrigants. This finding is in agreement with the results reported in previous studies.[32,67] This may be due to the combined actions of CHX and the detergent(surface active agent: N-Cetyl-N, N, N-trimethylammonium bromide) present in QMix. The rationale of adding a surface active agent in QMix is to lower surface tension and increase wettability,[86] which also enables better penetration of irrigant into the root canal. Chlorhexidine also has a surfactant molecule which increases the dentin surface free energy.[86] Smear layer tends to act as a barrier preventing diffusion of sealer into the dentinal tubule. The presence of EDTA in QMix improves wettability of sealer by removing smear layer and exposing dentinal tubules. [50]

Results of the present study show that final rinse with a chelating agent enhanced the bond strength of the AH Plus when compared to the sodium hypochlorite group. This could be attributed to two reasons: 1) Smear layer removal procedures allow the sealer penetration into the dentinal tubules and thus could increase the dentin bond strength of resin based sealer as well as an enhanced seal.2) The suggested mechanism of bonding of AH Plus to the organic phase of the root dentine, most likely in the collagen network.[8]

Comparing EDTA, Citric acid and QMix, similar bond strength values were found. **Aranda-Garcia *et al.*[16] and Dai *et al*.**[17] observed that protocols using NaOCl during preparation, and EDTA or QMix as final rinse showed similar smear layer and debris removal capacity. **Takeda et al** observed no difference in smear layer removal capacity of 17% EDTA and 6% citric acid.[87] Similar smear layer removal capacity of the irrigants may allow comparable sealer penetration, justifying the similar results of push-out bond strength of the present study. **Zehnder et al[23]** stated that there was no increase in calcium chelating ability from instrumented root canals when the surface tension of chelator solutions was lowered with wetting agents which also might account for the similar bond strengths. Similar to our results, **Aranda-Garcia *et al*.**[16] and **Leal et al**[88] observed similar push-out bond strength values when EDTA and QMix were used as final irrigant, after the use of NaOCl.

Contrary to the results of the present study, **Stelzer et al**[53]**and Tuncel et al**[56] reported that EDTA did not enhance the bond strength of AH plus sealer.

LIMITATIONS OF THE STUDY:

- Oral cavity temperature, surface roughness of the instrumented root canal surface, method of sealer placement differ in clinical situations when compared to the experimental scenario of this in vitro study. These factors might affect the contact angle values in vivo.
- Studies to evaluate the influence of wettability on sealer penetration are needed.

Improved wettability may enhance the spreading of the sealer circumferentially over the root dentin surface. It may also enhance the penetration of sealers into lateral canals thus contributing to a three dimensional obturation. Future research should focus on the effect of wettability on sealer penetration and three dimensional obturation of root canals.

In summary, QMix improves the wettability of AH plus. However its effect on bond strength of AH plus is similar to other chelating agents used in the study. The improved wettability may enhance the sealer penetration which might enhance the antimicrobial effect of the sealer.

Summary and Conclusion

SUMMARY AND CONCLUSION

The aim of the study was to compare the effect of various final irrigation regimen on the wettability and push out bond strength of a epoxy resin root canal sealer AH plus.

To assess wettability, 20 premolars were cross-sectioned into two separate 1-mm-thick dentine discs thus obtaining 40 dentin discs. Dentin discs were then divided randomly into 4 groups (n = 10) depending on the irrigation regimen:

- Group I: 5 ml of 3% NaOCl.
- Group II: 5 ml of 3% NaOCl + 5 ml of 17% EDTA.
- Group III: 5 ml of 3% NaOCl + 5 ml of 10% citric acid.
- Group IV: 5 ml of 3% NaOCl + 5 ml of Q mix.

The contact angle was measured using a Dynamic Contact Angle Analyzer. Images of the droplets were then captured using the Digidrop software to determine the static contact angles made by the sealers on root canal dentine.

To assess push out bond strength, eighty extracted premolars with single canal were collected and decoronated at cementoenamel junction (CEJ). Biomechanical preparation was performed with ProTaper Universal NiTi rotary instrument. Teeth were randomly divided into four groups based on irrigation regimen similar to grouping in wettability as mentioned above. Obturation was done with AH plus sealer and guttapercha cones by cold lateral condensation. All specimens were stored at 37 degree Celsius, 100% humidity for 48 hours to ensure complete setting of the sealer.

One mm slices of mid-root dentin were made. The root filling in each section was subjected to universal testing machine at a crosshead speed of 1mm/minute. Load was applied in apico-coronal direction until bond failure occurred. The maximum failure load was recorded in Newtons, and push-out bond strength was calculated in megapascals (MPa).

Within the limitations of this in vitro study, QMix enhanced the wettability of AH plus root canal sealer to dentin. Chelating agent enhanced the bond strength of the AH Plus to root dentin. However there was no significant difference in bond strength of AH plus when root canal was irrigated with QMix compared to EDTA and Citric acid. The enhanced wettability of AH plus by QMix may improve the sealer penetration into root dentinal tubules which in turn may affect the sealing ability and antimicrobial activity of the sealer and this warrants further research.

Bibliography

BIBLIOGRAPHY

1. Sousa-Neto MD, Coelho FI, Marchesan MA, Alfredo E, Silva- Sousa YTC. *In vitro* study of the adhesion of an epoxy based sealer to human dentine submitted to irradiation with Er:YAG and Nd:YAG lasers. Int Endod J. 2005;38:866-870.

2. Sousa-Neto MD, Passarinho-Neto JG, Carvalho-Júnior JR, Cruz-Filho AM, Pécora JD, Saquy PC. Evaluation of the effect of EDT A, EGT A and CDT A on dentin adhesiveness and microleakage with different root canal sealers. Braz Dent J. 2002;13:123-128.

3. Schwartz RS. Adhesive dentistry and endodontics Part 2: bonding in the root canal system-the promise and the problems: a review. J Endod. 2006;32(12):1125-34.

4. De-Deus G, Di Giorgi K, Fidel S, Fidel RA, Paciornik S. Push-out bond strength of Resilon/Epiphany and Resilon/ Epiphany self-etch to root dentin. J Endod. 2009; 35(7): 1048–1050.

5. Gesi A, Raffaelli O, Goracci C, Pashley DH, Tay FR, Ferrari M. Interfacial strength of Resilon and gutta-percha to intraradicular dentin. J Endod. 2005;31(11):809-13.

6. Nunes VH, Silva RG, Alfredo E, Sousa-Neto MD, Silva-Sousa YT. Adhesion of Epiphany and AH Plus sealers to human root dentin treated with different solutions. Braz Dent J.2008;19(1): 46-50

7. De-Deus G, Namen F, Galan JJ, Zehnder. Soft chelating irrigation protocol optimizes bonding quality of Resilon/ Epiphany root fillings. J Endod. 2008;34(6):703-5.

8. Fisher MA, Berzins DW, Bahcall JK. An in vitro comparison of bond strength of various obturation materials to root canal dentin using a push-out test design. J Endod. 2007;33(7):856-8.

9. Mai S, Kim YK, Arola DD et al. Differential aggressiveness of ethylenediaminetetraacetic acid in causing canal wall erosion in the presence of sodium hypochlorite. J Dent. 2010;38(3):201-6.

10. Zhang K, Kim YK, Cadenaro M et al. Effects of different exposure times and concentrations of sodium hypochlorite/ethylenediaminetetraacetic acid on the structural integrity of mineralized dentin. J Endod. 2010 ;36(1):105-9.

11. LottantiS, Gautschi H, Sener B, Zehnder M. Effects of ethylenediaminetetraacetic, etidronic and peracetic acid irrigation on human root dentine and the smear layer. Int Endod J. 2009;42(4):335-43.

12. Prati C, Biagini G, NucciC,Castaldini C, Zucchini C. Effects of chemical pretreatments on dentin bonding. Am J Dent. 1990;3(5):199-206.

13. Hebling J, Pashley DH, Tjaderhane L, Tay FR. Chlorhexidine arrests subclinical degradation of dentin hybrid layers in vivo. J Dent Res. 2005;84(8):741-6.

14. Chandrasekhar V, Amulya V, Rani VS, Prakash TJ, Ranjani AS, Gayathri CH. Evaluation of biocompatibility of a new root canal irrigant QMixTM 2in1–an in vivo study. J Conserv Dent. 2013; 16(1): 36–40.

15. Stojicic S, Shen Y, Qian W, Johnson B, Haapasalo M. Antibacterial and smear layer removal ability of a novel irrigant, QMiX. Int Endod J. 2012; 45(4): 363–71.

16. Aranda-Garcia AJ, Kuga MC, Vitorino KR *et al*. Effect of the root canal final rinse protocols on the debris and smear layer removal and on the push-out strength of an epoxy-based sealer. Microsc Res Tech. 2013;76(5): 533–7.

17. Dai L, Khechen K, Khan S et al. The effect of QMix, an experimental antibacterial root canal irrigant, on removal of canal wall smear layer and debris. J Endod. 2011;37(1): 80–4.

18. Eliot C, Hatton JF, Stewart GP et al. The effect of the irrigant QMix on removal of canal wall smear layer: an ex vivo study. Odontology. 2014;102(2): 232–40.

19. Delivanis PD, Mattison GD, Mendel RW. The survivability of F43 strain of Streptococcus sanguis in root canals filled with gutta-percha and Procosol cement. J Endod. 1983;9(10):407-10.

20. Drummond JL, Sakaguchi RL, Racean DC, Wozny J, Steinberg AD. Testing mode and surface treatment effects on dentin bonding. J Biomed Mater Res. 1996;32(4):533-41.

21. Scelza MF, Teixeira AM, Scelza P. Decalcifying effect of EDTA-T, 10% citric acid, and 17% EDTA on root canal dentin. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2003;95(2):234–6.

22. Goracci C, Tavares AU, Fabianelli A, Monticelli F, Raffaelli O, Cardoso PC, Tay F, Ferrari M. The adhesion between fiber posts and root canal walls: comparison between microtensile and push-out bond strength measurements. Eur J Oral Sci. 2004;112(4):353– 361.

23. Zehnder M, Schicht O, Sener B, Schmidlin P. Reducing surface tension in endodontic chelator solutions has no effect on their ability to remove calcium from instrumented root canals. J Endod. 2005;31(8):590–592.

24. DoganBuzoglu H, Calt S, Gumusderelioglu M. Evaluation of the surface free energy on root canal dentine walls treated with chelating agents and NaOCl. Int Endod J. 2007;40(1):18-24.

25. Jainaen A, Palamara JEA, Messer HH. Push-out bond strengths of the dentine–sealer interface with and without a main cone. Int Endod J. 2007;40(11):882–890.

26. Teixeira CS, Alfredo E, Thome LH, Gariba-Silva R, Silva-Sousa YT, Sousa-Neto MD. Adhesion of an endodontic sealer to dentin and gutta-percha: shear and push-out bond strength measurements and SEM analysis. J Appl Oral Sci. 2009;17(2):129–35.

27. Balguerie E, van der Sluis L, Vallaeys K, Gurgel-Georgelin M, Diemer F. Sealer penetration and adaptation in the dentinal tubules: a scanning electron microscopic study. J Endod. 2011;37(11):1576-9.

28. De-Deus G, Branda˜ o MC, Leal F, Reis C, Souza EM, Luna AS, Paciornik S, Fidel S. Lack of correlation between sealer penetration into dentinal tubules and sealability in nonbonded root fillings. Int Endod J. 2012;45(7):642–651.

29. Silva PV, Guedes DFC, Nakadi FV, Pe´cora JD, Cruz-Filho AM. Chitosan: a new solution for removal of smear layer after root canal instrumentation. Int Endod J.2013; 46(4):332–338.

30. Tummala M, Chandrasekhar V, Rashmi AS, Kundabala M, Ballal V. Assessment of the wetting behavior of three different root canal sealers on root canal dentin. J Conserv Dent. 2012;15(2):109-12.

31. Vilanova WV, Carvalho-Junior JR, Alfredo E, Sousa- Neto MD, Silva-Sousa YTC. Effect of intracanal irrigants on the bond strength of epoxy resin-based and methacrylate resin- based sealers to root canal walls. Int Endod J. 2012;45(1):42–48.

32. Ballal NV, Tweeny A, Khechen K, Prabhu KN, Satyanarayan, Tay FR. Wettability of root canal sealers on intraradicular dentine treated with different irrigating solutions. J Dent. 2013;41(6):556-60.

33. López GL, de la Casa ML, Torres PL, del MilagroSáez M, López ME. Effect of combinations of irrigating solutions on dentine wettability. EndodPrac Today. 2013;7(1):65-69.

34. Alkahtani A, Alkahtany SM, Mahmood A, Elsafadi MA, Aldahmash AM, Anil S. Cytotoxicity of QMix™ endodontic irrigating solution on human bone marrow mesenchymal stem cells. BMC Oral Health. 2014;14:27

35. Bohn S, Ilie N. Wetting behaviour of silicone- and resin- based root canal sealers. Int Endod J. 2014;47(6):542–549.

36. Das A, Kottoor J, Mathew J, Kumar S, George S. Dentine microhardness changes following conventional and alternate irrigation regimens: An *in vitro* study. J Conserv Dent. 2014;17(6):546-9.

37. Elnaghy AM. Effect of QMixirrigant on bond strength of glass fibre posts to root dentine. Int Endod J. 2014;47(3):280–289.

38. Taneja S, Kumari M, Anand S. Effect of QMix, peracetic acid and ethylenediaminetetraacetic acid on calcium loss and microhardness of root dentine. J Conserv Dent. 2014;17(2):155-8.

39. Arslan H, Uygun AD, Keskin A, Karatas E, Seckin F, Yıldırım A. Evaluation of orange-brown precipitate formed in root canals after irrigation with chlorhexidine and QMix and spectroscopic analysis of precipitates produced by a mixture of chlorhexidine/NaOCl and QMix/NaOCl. Int Endod J. 2015; 48(12):1199–1203.

40. Elakanti S, Cherukuri G, Rao VG, Chandrasekhar V, Rao AS, Tummala M. Comparative evaluation of antimicrobial efficacy of QMixTM 2 in 1, sodium hypochlorite, and chlorhexidine against *Enterococcus faecalis* and *Candida albicans*. J Conserv Dent. 2015;18(2):128-31.

41. Jardine AP, Rosa RA, Santini MF, Wagner M, Reis So MV, Kuga MC et al. The effect of final irrigation on the penetrability of an epoxy resin-based sealer into dentinal tubules: a confocal microscopy study. Clin Oral Invest. 2016;20(1):117–123

42. Mohan RP, Pai AR. The comparison between two irrigation regimens on the dentine wettability for an epoxy resin based sealer by measuring its contact angle formed to the irrigated dentine. J Conserv Dent. 2015;18(4):275-8.

43. Neelakantan P, Sharma S, Shemesh H, Wesselink PR. Influence of Irrigation Sequence on the Adhesion of Root Canal Sealers to Dentin: A Fourier Transform Infrared Spectroscopy and Push-out Bond Strength Analysis. J Endod. 2015;41(7):1108–1111.

44. Uzunoglu E, Turker SA, Karahan S. The Effect of Increased Temperatures of QMix and EDTA on the Push-out Bond Strength of an Epoxy-resin Based Sealer. J ClinDiagn Res. 2015; 9(7):ZC98–ZC101.

45. Balić M, Lucić R, Mehadžić K, Bago I, Anić I, Jakovljević S, Plečko V. The efficacy of photon-initiated photoacoustic streaming and sonic-activated irrigation combined with QMiX solution or sodium hypochlorite against intracanal E. faecalis biofilm. Lasers Med Sci. 2016;31(2):335-42.

46. Souza MA, Montagner A, Lana DL, Vidal CM, Farina AP, Cecchin D. Comparative evaluation of the retaining of QMix and chlorhexidine formulations on human dentin: a chemical analysis. Clin Oral Investig. 2016;30.

47. Azar NG, Heidari M, Bahrami ZS, Shokri F. *In vitro* cytotoxicity of a new epoxy resin root canal sealer. J Endod. 2000;26(8):462-5.

48. Dogan H, Oalt S. Effects of chelating agents and sodium hypochlorite on mineral content of root dentin. J Endod. 2001;27(9):578–80.

49. Khedmat S, Shokouhinejad N. Comparison of the efficacy of three chelating agents in smear layer removal. J Endod. 2008; 34(5): 599–602

50. Hu X, Ling J, Gao Y. Effects of irrigation solutions on dentin wettability and roughness. J Endod. 2010;36(6):1064-7.

51. Dai L, Khechen K, Khan S, Gillen B, Loushine BA, Wimmer CE, et al. The effect of QMix, an experimental antibacterial root canal irrigant, on removal of canal wall smear layer and debris. J Endod. 2011;37(1):80-4.

52. Neelakantan P, Subbarao C, Subbarao CV, De-Deus G, Zehnder M. The impact of root dentine conditioning on sealing ability and push-out bond strength of an epoxy resin root canal sealer. Int Endod J.2011; 44(6): 491–498.

53. Stelzer R, Schaller HG, Gernhardt CR. Push-out bond strength of RealSeal SE and AH Plus after using different irrigation solutions. J Endod. 2014;40(10):1654-7.

54. Gründling GL, de Melo TAF, Montagner F, Scarparo RK, Vier-Pelisser FV. QMix irrigant reduces lipopolysacharide (LPS) levels in an in vitro model. J. Appl. Oral Sci. 2015;23(4): 431-435.

55. Kaushik M, Sheoran K, Reddy P, Roshni, Narwal P. Comparison of the effect of three different irrigants on the contact angle of an epoxy resin sealer with intraradicular dentin. Saudi Endod J. 2015;5(3):166-70

56. Tuncel B, Nagas E, Cehreli Z, Uyanik O, Vallittu P, Lassila L. Effect of endodontic chelating solutions on the bond strength of endodontic sealers. Braz Oral Res. 2015;29(1):1-6.

57. Centers for Disease Control and Prevention. 2003 CDC infection control recommendations for dental health-care settings. Compend Contin Educ Dent. 2004;25:43-8,50-3.

58. Kohn WG, Harte JA, Malvitz DM, Collins AS, Cleveland JL, Eklund KJ. Guidelines for infection control in dental health care set- tings--2003. J Am Dent Assoc. 2004;135:33-47.

59. Sjogren U, Hagglund B, Sundqvist G, Wing K. Factors affecting the long term results of endodontic treatment. J Endod.1990;16(10):498–504.

60. Lee KW, Williams MC, Campus JJ, Pashley DH. Adhesion of endodontic sealers to dentin and gutta-percha. J Endod. 2002;28(10):684–8.

61. Erickson RL. Surface interactions of dentin adhesive materials. Oper Dent 1992;5: 81–94.

62. Eick JD, Gwinnett AJ, Pashley DH, et al. Current concepts on adhesion to dentin. Crit Rev Oral Biol Med. 1997;8(3):306–35.

63. Young T. An Essay on the Cohesion of Fluids. Phil. Trans. R. Soc. Lond. 1805; 95:65-87.

64. Erbil HY. Contact angle of liquid drops on solids. In: Surface Chemistry: Of Solid and Liquid Interfaces. Oxford, UK: John Wiley and Sons; 2009:p. 308–337.

65. Von Fraunhofer JA. Adhesion and cohesion. Int J of Dent. 2012 : 2012 : 951324

66. Huntsberger JR. Surface energy, wetting and adhesion. J Adhes.1981;12(1):3–12.

67. Neelakantan P, Varughese AA, Sharma S, Subbarao CV, Zehnder M, De-Deus G. Continuous chelation irrigation improves the adhesion of epoxy resin-based root canal sealer to root dentin. Int Endod J. 2012;45(12):1097–102.

68. Hashem AA, Ghoneim AG, Lutfy RA, Fouda MY. The effect of different irrigating solutions on bond strength of two root canal-filling systems. J Endod. 2009; 35(4): 537– 40.

69. Van Meerbeek B, Lambrecht P, Inokoshi S, et al. Factors affecting adhesion to mineralized tissues. Oper Dent 1992;5:111–24.

70. Zehnder M. Root canal irrigants. J Endod. 2006;32(5):389-98.

71. Crincoli V, Scivetti M, Di Bisceglie MB, Pilolli GP, Favia G. Unusual case of adverse reaction in the use of sodium hypochlorite during endodontic treatment: a case report. Quintessence Int. 2008;39(2):e70-3.

72. Haapasalo M, Shen Y, Qian W, Gao Y. Irrigation in endodontics. Dent Clin North Am. 2010;54(2):291-312.

73. Okino LA, Siqueira EL, Santos M, Bombana AC, Figueiredo JA. Dissolution of pulp tissue by aqueous solution of chlorhexidinedigluconate and chlorhexidinedigluconate gel. Int Endod J. 2004;37(1), 38–41.

74. Roggendorf M. BayerischesZahnärzteblatt. Sept. München Germany, Bavarian Dental Journal 2004. p. 32-4.

75. Shafrin EG, Zisman WA. Constitutive relations in the wetting of low energy surfaces and the theory of the retraction method of preparing monolayers. The J Phys Chem. 1960;64(5):519–24.

76. Zisman WA. Relation of the Equilibrium Contact Angle to Liquid and Solid Constitution. In: Contact Angle, Wettability and Adhesion;Advances in chemistry series, American chemical society: Washington, D.C.,1964;Vol 43,1-51.

77. Kurtz JS, Perdigao J, Geraldeli S, Hodges JS, Bowles WR. Bond strengths of tooth-colored posts, effect of sealer, dentin adhesive, and root region. Am J Dent. 2003;16:31A-36A.

78. Rosales JI, Marshal GW, Marshal SJ, et al. Acid-etching and hydration influence on dentin roughness and wettability. J Dent Res 1999;78(9):1554–1559.

79. Combe EC, Owen BA, Hodges JS. A protocol for determining the surface free energy of dental materials. Dent Mater. 2004;20(3):262–268.

80. Nakashima K, Terata R. Effect of pH modified EDTA solution to the properties of dentin. J Endod. 2005;31(1):47-9.

81. Attal JP, Asmussen E, Degrange M. Effects of surface treatment on the free surface energy of dentin. Dent Mater. 1994;10(4):259-64.

82. Good RJ, Koo MN. The effect of drop size on contact angle. J Colloid Interface Sci.1979;71(2):283–92.

83. Akinmade AO, Nicholson JW. Glass-ionomer cements as adhesives. Part I. Fundamental aspects and their clinical relevance. J Mater Sci: Mater Med 1993;4(2):95–101.

84. Tay FR, Gutmann JL, Pashley DH. Microporous, demineralized collagen matrices in intact radicular dentin created by commonly used calcium-depleting endodontic irrigants. J Endod. 2007;33(9):1086–90.

85. Saleh IM, Ruyter IE, Haapasalo M, Ørstavik D. The effects of dentine pretreatment on the adhesion of root canal sealers. Int Endod J. 2002;35(10):859–66.

86. Giardino L, Ambu E, Becce C, Rimondini L, Morra M. Surafce tension comparison of four common root canal irrigants and two new irrigants containing antibiotic. J Endod. 2006;32(11):1091-3.

87. Takeda.FH, Harashima T, Kimora Y, Matsumoto K. A comparative study of the removal of smear layer by three endodontic irrigants and two types of laser. Int Endod J. 1999; 32(1): 32-39.

88. Leal F, Simão RA, Fidel SR, Fidel RA, do Prado M. Effect of final irrigation protocols on push-out bond strength of an epoxy resin root canal sealer to dentin .Aust Endod J. 2015;41(3):135-9.